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JOURNAL OF NUCLEAR MEDICINE, vol. 24,
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tential of liposomes loaded with In-
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BIOCHEMISTRY, vol. 20, no. 14, 1981, pages 4229-4238, American Chemical Society, New York, US; F.J. MARTIN et al.:

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JOURNAL OF NUCLEAR MEDICINE, vol. 25, no. 4, 13th April 1984, New York, US; J.A. KOUTCHER et al.: "Contrast agents and spectroscopic probes in NMR"

RADIOLOGY, vol. 47, no. 3, June 1983, pages 781-788, Easton, US; R.C. BRASCH et al.: "Work in progress: methods of contrast enhancement for NMR imaging and potential applications"

Description

Field of the Invention

5 The invention described herein relates to enhanced contrast in nuclear magnetic resonance (NMR) imaging through the use of a paramagnetic material in association with micellular particles such as phospholipid vesicles.

10 Description of Prior Art

NMR imaging of humans is fast becoming a major diagnostic tool. Resolution is now on a par with X-ray CT imaging, but the key advantage of NMR is its ability to discriminate between tissue types (contrast) on the basis of differing NMR relaxation times, T_1 and T_2 . Because nuclear relaxation times can be strongly affected by paramagnetic ions such as Mn(II) and Gd(III) or stable free radicals, these materials have been explored to determine their ability to provide further contrast, specifically to test whether they alter water proton T_1 and T_2 values in excised animal organs and in live animals; see, for example, Mendonca Dias et al, The Use of Paramagnetic Contrast Agents in NMR Imaging, Absts. Soc. Mag. Res. Med., 1982, pages 103, 104; Brady et al, Proton Nuclear Magnetic Resonance Imaging of Regionally Ischemic Canine Hearts: effects of Paramagnetic Proton Signal Enhancement, Radiology, 1982, 144, pages 343-347; and Brasch et al, Evaluation of Nitroxide Stable Free Radicals for Contrast Enhancement in NMR Imaging, Absts. Soc. Mag. Res. Med., 1982, pages 25, 26; Brasch, Work in Progress: Methods of Contrast Enhancement for NMR Imaging and Potential Applications, Radiology, 1983, 147, p. 781-788; and Grossman et al, Gadolinium Enhanced NMR Images of experimental Brain Abscess, J. Comput. Asst. Tomogr., 1984, 8, p. 204-207. Results reported show that contrast is enhanced by a variety of paramagnetic agents.

25 However, useful compounds, due to the nature of the candidate paramagnetic materials, may be toxic at the concentrations required for optimal effect, and finding contrast agents for which the toxicity is low enough to make possible their eventual use in medical diagnosis is regarded as the most serious and difficult problem in the field, Mendonca Dias et al, The Use of Paramagnetic Contrast Agents in NMR Imaging, Absts. Soc. Mag. Res. Med., 1982, pages 105, 106. The invention described herein is thus designed to reduce toxicity and increase the utility of NMR contrast agents by associating a paramagnetic material with a micellular particle having properties tailored to the unique demands of NMR imaging.

Another significant problem which must be addressed is that the maximum tissue volume occupied by micelles such as vesicles generally does not exceed about 0.1%, which means that the micelle must be capable of affecting an image with a very small volume percentage. In this regard, however, paramagnetic NMR contrast agents differ fundamentally from contrast agents used as X-ray absorbers, gamma ray emitters or the like in other imaging modalities in which the signal or attenuation is simply proportional to the number per unit volume, no matter how they are chemically bound or entrapped. In NMR, the agent (ion or stable free radical) acts to increase the relaxation rate of bulk water protons surrounding the free electron spin. The phenomenon depends on rapid exchange of water on and off an ion or rapid diffusion of water past an organic free radical. In such case, the net relaxation rate is a weighted average for free and bound water.

Encapsulation of the paramagnetic material within a phospholipid vesicle, as in one preferred form of this invention, would seem to deny access of the paramagnetic agent to all but the entrapped water, typically less than 0.1% of the total volume. Under such conditions, the NMR image would not be altered detectably by the presence of vesicle-encapsulated contrast agent. Only if water exchanges sufficiently rapidly across the bilayer is the relaxation rate of the bulk water enhanced, Andrasko et al, NMR Study of Rapid Water Diffusion Across Lipid Bilayers in Dipalmitoyl Lecithin Vesicles, Biochem. Biophys. Res. Comm., 1974, 60, p. 813-819. The present invention addresses this problem by providing a formulation of micelle and paramagnetic material that simultaneously maximizes micelle stability while permitting adequate rates of water exchange across the membrane.

Phospholipid vesicles are known to concentrate in certain tissues, so additional enhancement will come from tissue specificity. For example, phospholipid vesicles have been observed to accumulate in implanted tumors of mice, Proffitt et al, Liposomal Blockade of the Reticuloendothelial System: Improved Tumor Imaging with Small Unilamella Vesicles, Science, 1983 220 p. 502-505, Proffitt et al, Tumor-Imaging Potential of Liposomes Loaded with In-111-NTA: Biodistribution in Mice, Journal of Nuclear Medicine, 1983, 24, p. 45-51.

The invention also extends the use of micellular particles as contrast agent carriers to applications

where the micelles are attached to antibodies. While it has been reported that a selective decrease in T_1 relaxation times of excised heart may be obtained using manganese-labeled monoclonal antimyosin antibody, Brady, et al, Selective Decrease in the Relaxation Times of Infrared Myocardium with the Use of a Manganese-Labeled Monoclonal Antibody, Soc. Magn. Res. Med., Works in Progress, Second Annual Meeting, 1983, p. 10, heretofore, due in large measure to considerations such as toxicity referred to above, the practical use of such antibodies has been significantly restricted. With the present invention, however, increased sensitivity is obtained and specificity is maintained by attachment of antibody to the surface of micellular particles. The antibodies provide high specificity for cell or tissue types, while the attached vesicle agent carriers amplify the NMR contrast enhancement over what can be achieved with ions bound to antibody alone.

SUMMARY OF THE INVENTION

The invention described herein is directed to preparations of micellular particles such as small unilamellar vesicles, with which a paramagnetic material is associated, typically paramagnetic compounds enclosed within the vesicles. The vesicles may or may not have antibodies, such as antimyosin, or antifibrin, attached to the surface or have other surface modifications for which there are specific cell receptors in certain tissue.

Examples of vesicle constituents are phospholipids such as distearoylphosphatidylcholine (DSPC), dipalmitoylphosphatidylcholine (DPPC), and dimyristoylphosphatidylcholine (DMPC). Examples of paramagnetic materials are salts of transition metals and the lanthanide and actinide series of the periodic table such as Gd(III), Mn(II), Cu(II), Cr(III), Fe(II), Fe(III), Co(II), Er(III), nickel(II) and complexes of such ions with diethylenetriaminepentaacetic acid (DTPA), ethylenediaminetetraacetic acid (EDTA) and other ligands. Other paramagnetic compounds include stable free radicals such as organic nitroxides.

Vesicle-encapsulated contrast agents may be prepared by forming the lipid vesicles in an aqueous medium containing the paramagnetic agent by any suitable means such as sonication, homogenization, cholate dialysis and the like, and then freeing the vesicles of external agent by ultrafiltration, gel filtration or similar method. Moreover, the internal solution of the paramagnetic material may be altered readily to maximize the relaxation rate per unit of agent as for example, by formulation with a charged polymeric material such as poly-L-Lysine.

DETAILED DESCRIPTION

Definitions and Abbreviations

As used herein, "micellular particle" and "micelles" refer to particles which result from aggregations of amphiphilic molecules. In this invention, preferred amphiphiles are biological lipids.

"Vesicle" refers to a micelle which is in a generally spherical form, often obtained from a lipid which forms a bilayered membrane and is referred to as a "liposome". Methods for forming these vesicles are, by now, very well known in the art. Typically, they are prepared from a phospholipid, for example, distearoyl phosphatidylcholine or lecithin, and may include other materials such as neutral lipids, and also surface modifiers such as positively or negatively charged compounds. Depending on the techniques for their preparation, the envelope may be a simple bilayered spherical shell (a unilamellar vesicle) or may have multiple layers within the envelope (multi-lamellar vesicles). DSPC = distearoyl phosphatidylcholine

Ch = cholesterol

DPPC = dipalmitoylphosphatidylcholine

DMPC = dimyristoylphosphatidylcholine

DTPA = diethylenetriaminepentaacetic acid

EDTA = ethylenediaminetetraacetic acid

SUV = small unilamellar vesicles

Materials and Preparation of Micelles

Complexes of paramagnetic compounds were prepared in deionized water or in a buffer of 4.0 mM Na_2HPO_4 , 0.9% (by weight) NaCl, pH 7.4 (PBS).

Gd(III)-citrate. A stock solution of 1.0 mM Gd(III)-10.0 mM citrate was made by dissolving 10.0 μ moles $\text{GdCl}_3 \cdot 6\text{H}_2\text{O}$ (99.999%, Aldrich) in 9 ml deionized water and adding 100 μ moles $\text{Na}_3\text{citrate}$ (analytical reagent, Mallinckrodt). The pH was adjusted to neutrality and the volume brought to 10.0 mL in a volumetric flask.

Mn(II)-citrate. A stock solution of 1.0 mM Mn(II)-10.0 mM citrate was made by adding 10.0 μ moles $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (Baker analyzed) and 100 μ moles sodium citrate to 9 ml water. The solution was neutralized and made up to 10.0 ml in a volumetric flask.

Gd(III)-DTPA. A stock solution of 200 mM Gd(III)-210 mM DTPA was made by dissolving 2.10 mmoles DTPA in minimum 6 N NaOH in a 10 ml volumetric flask. 2.0 mmoles $\text{GdCl}_3 \cdot 6\text{H}_2\text{O}$ were added and the pH adjusted to 7.4 with 6 N NaOH, after which the sample was made up to 10.0 ml in the flask.

La(III)-DTPA. A stock solution of 200 mM La(III)-210 mM DTPA was made up in a manner analogous to the Gd(III)-DTPA stock using $\text{LaCl}_3 \cdot 7\text{H}_2\text{O}$ (99.999%, Aldrich).

Er(III)-EDTA. Stock solutions were prepared in a manner analogous to Gd(III)-DTPA.

Poly-L-lysine hydrobromide of approximate average molecular weights 25,000 and 4,000 were obtained from Sigma Chemical Co.

Cholesterol (98%) was from Mallinckrodt. DSPC was synthetic material from Cal-Biochem.

DSPC/cholesterol vesicle-encapsulated NMR contrast agent. 16 mg DSPC and 4 mg cholesterol were dissolved in 2 ml CHCl_3 . 10 μ l of a solution of 0.16 mM cholesterol [^{14}C] (56.5 mCi/mmol) in CHCl_3 were added for purposes of quantitating lipid concentrations in the final preparations. The lipid solution was evaporated to dryness in a vacuum dessicator and stored in the same, if not used immediately.

Small unilamellar vesicles (SUV) were formed in a solution of 200 mM Gd(III)-DTPA by adding 2.0 ml of the stock ion complex to the dried lipid tube. The mixture was complex to the dried lipid tube. The mixture was sonicated using an Ultrasonics, Inc. probe with a microtip at a power level of 56 W. The tube was cooled by partial immersion in a water bath, and N_2 was flowed over the sample during sonication. Total time of sonication was 15 min. or more until the solution was slightly opalescent.

Paramagnetic agent outside the vesicles was separated from the SUVs by passage through columns of Sephadex G-50 swollen in PBS, that had been loaded into 3 ml plastic syringe bodies and precentrifuged. The vesicle solution was placed at the top of the syringe and centrifuged with a glass tube positioned to collect the eluate. 300 μ l PBS was used to elute the vesicles from the columns. The procedure was repeated a total of 3 times to reduce the outside concentration of free agent and to exchange it for PBS.

Vesicle concentration in the final preparation was measured by counting an aliquot of the solution in the scintillation counter, using a standard cocktail. Average vesicle size was measured in a laser particle sizer Model 200 (Nicomp Instruments). The vesicle size was measured to be $600 \pm 100 \text{ \AA}$ (cm^{-1}) in all experiments.

NMR Relaxation time measurements.

Unless otherwise indicated, measurements of T_1 and T_2 were made at 20 MHz with a pulsed NMR spectrometer (IBM PC/20) interfaced to a microcomputer (IBM PC). T_1 was measured by the inversion-recovery method (Farrar, T.C., Becker, E.D., Pulse and Fourier Transform NMR, 1971, Academic Press, New York.) and T_2 by the Carr-Purcell sequence (Carr, H.Y., Purcell, E.H., Effects of Diffusion on Free Precession in NMR Experiments, Phys. Rev., 1954, 94, p. 630-633.), as modified by Meiboom and Gill (Meiboom S., Gill D., Modified Spin-Echo Method for Measuring Nuclear Relaxation Times, Rev. Sci. Instrum., 1958, 29, p. 688-691.) Least-squares best fits of the data to single exponential recoveries were done automatically by the computer. Values of T_1 and T_2 reported are for a probe temperature of 38°C. T_1 values are estimated to have an experimental uncertainty of $\pm 10\%$ and a reproducibility of $\pm 5\%$. T_2 values are generally accurate to within $\pm 20\%$ and reproducible to $\pm 5\%$. This precision is sufficient clearly to demonstrate the effects claimed. Some values of T_1 were measured with a Praxis II NMR spectrometer operating at 10 MHz and a probe temperature of 25°C.

Animal Studies

EMT6 tumor tissue was transplanted subcutaneously into the flank of male Balb/c mice and allowed to grow for 10 days. On the 10th day, mice were injected i.v. with 200 μ l of vesicle solution or control buffer.

Mice were sacrificed at intervals, and the tumors were dissected. In some experiments liver and spleen were also dissected. The tissue was rinsed in PBS, lightly blotted, weighed, and wrapped in air tight plastic bags. NMR relaxation measurements were made within 1/2 hr of dissection to limit water loss and consequent changes in T_1 and T_2 .

5

Description of Drawings

Figure 1 is a plot of longitudinal relaxation rate as a function of added paramagnetic ion concentration. Aliquots of the stock solutions were added to PBS buffer. T_1 measurements were made at 10 MHz using the $90^\circ - 90^\circ$ method. Probe temperature was 25° . Concentration scale for Er-EDTA is in mM units while for Mn-citrate the units are μ M.

In Figure 2, the dependence of $1/T_1$ on added Gd ion in various forms is illustrated. Aliquots of stock solutions were added to water (Gd/citrate) or PBS (Gd/DTPA and Gd/DTPA in vesicles) to give the total concentration of ion indicated.

Figure 3 illustrates internal paramagnetic ion complex concentration effects on $1/T_1$ and $1/T_2$. DSPC/cholesterol vesicles were prepared with increasing concentrations of Gd-DTPA in PBS encapsulated inside. The lipid (vesicle) concentrations were all adjusted with PBS to be equal at 8.3 mg/ml total lipid final concentration.

Figure 4 illustrates relaxation rates of mouse tissue and tumors. Balb/c mice were injected with 200μ l of 200 mM Gd-DTPA in DSPC/cholesterol vesicles (10 mg/ml lipid) (Gd Ves), 200 mM La-DTPA in DSPC/cholesterol vesicles (La Ves), 2.0 mM Gd-DTPA in PBS (Gd Buf) or PBS(Buf). After 16 hrs, the mice were sacrificed and the tissues dissected. Relaxation times are the average for at least 3 animals.

Figure 5 shows the effect of added poly-L-lysine on relaxation rates of Gd-DTPA solutions. Dry weighed aliquots of poly-L-lysine were dissolved in 2.0 ml of 2.0 mM Gd-DTPA in H_2O . T_1 and T_2 were measured as described in the text.

Figure 6 illustrates the time course of $1/T_1$ for mouse tumors. Preparations of 10 mg/ml lipid vesicles containing 200 mM Gd-DTPA inside were injected (200μ l) into the tail vein of Balb/C mice having 10 day old EMT6 tumors from previous implants. The mice were sacrificed at intervals and T_1 of the tumors measured immediately after dissection. Controls were either no injection (0) or 200μ l of 2.0 mM Gd-DTPA in PBS (\square). Three separate experiments are collected in this graph. For the \circ and \square data, the points each represent T_1 for a single tumor. For the Δ data, 2 or 3 T_1 values were occasionally measured for a single tumor.

Referring now in more detail to the figures of drawing, the improved results of the present invention will be discussed. In Figure 1, relaxation rates of Er-EDTA and Mn citrate solutions are shown. Values of $1/T_1$ are plotted as a function of ion concentration at 10 MHz and 25° C. The average value of $1/T_1$ for mouse soft tissue is indicated on the graph. The concentration scale for Er-EDTA is millimolar while that for Mn-citrate is micromolar. Addition of 18 mM Er-EDTA complex to a PBS solution increases $1/T_1$ to the mouse tissue value of $2.4 s^{-1}$. The same relaxation rate is achieved with only 0.17 mM Mn-citrate complex. The weak complex of Mn is 100 times more efficient for relaxation enhancement than the strongly complexed Er-EDTA, reflecting the intrinsically stronger relaxation power of the Mn(II) ion as well as the greater accessibility of the Mn to water.

In Figure 2, the relaxation effects of Gd(III) are shown. At 20 MHz the addition of Gd-citrate to H_2O increases $1/T_1$ to a value of $8.1 s^{-1}$ at 1.0 mM. When complexed to DTPA, the ion has one-half the relaxation effect. This reduction occurs because of displacement of water binding sites by the DTPA functional groups, partly balanced by an increased rotational correlation time of the complex. With the Gd-DTPA complex encapsulated in DSPC-cholesterol vesicles, the solution $1/T_1$ is still increased to a value of $2.5 s^{-1}$ for 1.0 mM total Gd-DTPA. While less efficient than free Gd-DTPA per unit ion, the vesicles still have a substantial effect on water relaxation.

The effect of internal paramagnetic ion complex concentration on relaxation rates for vesicle-encapsulated Gd-DTPA is shown by Figure 3. $1/T_1$ and $1/T_2$ for vesicle solutions increase linearly up to 150 mM internal Gd-DTPA concentration.

$$\frac{1}{T_1} \text{ obsd} = \frac{Pb}{(T_1 b + \tau b)} + \frac{1}{T_1 a}, \text{ wherein } b$$

is for inside the vesicle and a is outside, τ b is the lifetime of water protons inside, $T_1 b$ is the net relaxation

time of water inside (made small by the paramagnetic agent), and P_b is the fraction of water inside the vesicle, predicts a linear dependence of $1/T_1$ on paramagnetic ion concentration until the value of $T_1 b$ becomes on the order of or less than τ . The results shown by Figure 3 suggest that up to 150 mM Gd-DTPA concentration, T_1 inside the vesicles is greater than the exchange lifetime, τ .

5 Relaxation effects on mouse tissue and tumors are illustrated in Figure 4. The T_1 values of Balb/c mouse liver, spleen, kidney and EMT6 tumor tissue are compared 16 hours after injection of paramagnetic agents or controls. Vesicle encapsulated Gd-DTPA promotes a significant reduction in T_1 for spleen and for EMT6 tumors compared to the control of the diamagnetic Lanthanide ion complex of La-DTPA in vesicles (spleen) or PBS buffer plus 2.0 mM Gd/DTPA. (tumor). In the case of Gd/DTPA-vesicle treated mice, the T_1 values averaged 17% less than controls without injected agent.

10 The foregoing data allow an estimate of the minimum Gd/DTPA or other paramagnetic species concentrations inside vesicles which provide contrast enhancement. There is a complex set of interrelating factors, such as proton exchange rate across membranes of the tumor cells, wash out rate of free Gd/DTPA from lipid vesicles, and altered rotational correlation time of the complex in a macromolecular environment, which contributes to the T_1 proton relaxation rate and subsequent contrast enhancement. The amount of accumulated vesicles in a particular tissue to be imaged dictates the minimum concentration of encapsulated paramagnetic material. For this Murine tumor model, it has been inferred that approximately 0.1% of the tumor volume is occupied by intact vesicles.

While the quantity of paramagnetic material to be encapsulated will vary, depending upon the specific material used as well as the factors mentioned above, in general, the paramagnetic material will be at least approximately 50 mM in the vesicle. The maximum quantity will be dictated by consideration of cost, toxicity and vesicle formulation, but ordinarily will not be above about 1 M encapsulated concentration.

Figure 5 illustrates the enhanced relaxation rates through addition of a polymer. The relaxation effect of Gd-DTPA can be enhanced by the addition of the positively charged polymer, poly-L-lysine. Figure 5 shows the result of adding poly-L-Lys of average MW 25,000 to a solution of 2.0 mM Gd-DTPA in H_2O . A 40% increase in relaxation rate $1/T_1$ is obtained and in 30% increase in $1/T_2$. The effect of added poly-L-lysine plateaus above 3 mg/ml showing a "weak binding" situation. This leveling off also shows that the increased relaxation rate is not due to an increase in viscosity, since the effect there would be linear in added poly-Lys over the whole concentration range. Smaller molecular weight poly-L-Lys is less effective on a weight basis. Gd-DTPA is a negatively charged complex which binds reversibly to the positive charge of the poly-Lys. The large size and consequent slow tumbling of the macromolecule made relaxation of the paramagnetic ion more efficient. This effect can be used by co-encapsulating Gd-DTPA and poly-Lys or some like positively charged macromolecule to increase the effect per unit ion of Gd and thus decrease net toxicity of the preparation.

35 Time course of relaxation effect on EMT6 tumors is shown in Figure 6. The maximal effect of vesicle-encapsulated Gd-DTPA is achieved 3-4 hrs after injection of the agent. The average effect at 4 hrs is approximately equal to that at 16 hrs post-injection, suggesting that a steady-state condition obtains where the rate of uptake by tumor is matched by loss of agent to the circulation.

Three different liposome formulations were tested at doses higher than used for data of Figure 6 for their relaxation effects on EMT6 tumors subcutaneously implanted in Balb/c mice. The mice were injected intravenously with the agent and then sacrificed at intervals. Tumor and liver T_1 values were measured within 1/2 hour of sacrifice of the animal. The results are set forth in Table I for tumors and in Table II for liver. Animals receiving only buffer had an average tumor T_1 value of 960 ± 41 ms ($n = 25$) and an average liver T_1 value of 392 ± 31 ms ($n = 24$). The tumor relaxation time decreased to 665 ± 28 ms ($n = 4$) at 24 hours post injection for the 1:1 DSPC/CHOL formulation, while the livers of these animals had average T_1 values of 370 ± 13 ms ($n = 4$). The T_1 change of 44% for the tumors is substantially larger than that for the liver (6%).

50 With many liposome formulations in common use, liver (and spleen) accumulate the largest fraction of the vesicle dose. The particular formulation of the present invention is thus far more specific for the tumor, at least in its effect on NMR relaxation times. The vesicle-encapsulated paramagnetic complex of the present invention accordingly fulfills the requirement of an NMR imaging contrast agent: that is, it leads to reduced values of T_1 in selected tissues. In this case, the original long T_1 of the tumor before contrast agent (average 960 ms) will leave the tumor dark in an NMR image, while, after injection of agent, the tumor would appear brighter in the scan.

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Table I. TUMOR RELAXATION RATEEMT6 Tumor in Flank of Balb/c Mouse
(10 day tumor growth)

Vesicle-encapsulated NMR Contrast Agent

Values are T_1 (in ms) \pm standard deviation

n = number of mice

Formulation *	Post-injection Time (hr)				Notes
	.1-2	2-5	5-8	24	
PBS Control	962 \pm 24 n = 9	974 \pm 50 n = 11	920 \pm 19 n = 4	926	Global Average 960 \pm 41 n = 25
25 DPPC/CHOL 2:1 Gd/DTPA 200 mM	869 \pm 28 n = 14	840 \pm 30 n = 13	845 \pm 40 n = 13		
30 Gd DSPC/CHOL 2:1 Gd DTPA 200 mM	812 \pm 45 n = 8	768 \pm 30 n = 8	769 \pm 28 n = 4		
35 DSPC/CHOL 1:1 Gd/DTPA 200 mM		710 \pm 19 n = 2	720 \pm 21 n = 3	665 \pm 28 n = 4	

* Injection volume = 250-300 μ l
Lipid concentration generally 20 mg/ml

Table II. LIVER RELAXATION RATE

Tumor bearing Balb/c Mouse

Post Contrast Agent Injection

Vesicle-encapsulated NMR Contrast Agent

Values are T_1 (in ms) \pm standard deviation

n = number of mice

Formulation *	Post-injection Time (hr)				Notes
	1-2	2-5	5-8	24	
PBS Control	400 \pm 39 n = 8	380 \pm 26 n = 12	411 \pm 6 n = 3	412 n = 1	Global Average 392 \pm 31 n = 24
DPSPC/CHOL 2:1 Gd/DTPA 200 mM	379 \pm 35 n = 11	377 \pm 29 n = 11	375 \pm 34 n = 11		
DPSPC/CHOL 2:1 Gd/DTPA 200 mM	349 \pm 17 n = 11	345 \pm 13 n = 11	379 \pm 29 n = 7		
DPSPC/CHOL 1:1 Gd/DTPA 200 mM		330 \pm 32 n = 2	342 \pm 11 n = 3	370 \pm 13 n = 4	

* Injection volume = 250-300 μ l
Lipid concentration generally 20 mg/ml

For an NMR imaging contrast agent to be most useful, it must yield the maximum increase of $1/T_1$ possible with minimum toxicity, and have specificity for tissue type. The invention provides these features. A macromolecular assembly can increase the relaxation effect per unit ion, as demonstrated by the effect of added poly-Lys on $1/T_1$ and $1/T_2$ of Gd-DTPA solutions (Figure 5). Low toxicity is gained by associating the normally toxic paramagnetic ion with a strong chelate in a macromolecular assembly (e.g. encapsulation in a vesicle) which keeps the ion out of circulation. NMR relaxation is enhanced by formulating the vesicle to maximize access of H_2O protons to the ion. This was accomplished as shown by the strong relaxation effect of encapsulated Gd-DTPA (Figure 2). Tissue specificity is provided by the complex nature of the micellar assembly for which biological recognition processes cause the macromolecule to distribute to certain sites. This is demonstrated for phospholipid vesicles by the differential influence on tissue relaxation

rates (Figure 4, tables I and II) and by the specific effect on tumor relaxation of Gd-DTPA encapsulated in vesicles versus approximately the same total concentration of Gd-DTPA free in solution (Figures 4 and 6).

It has been described herein that antibodies can be bound to vesicles to obtain tissue specificity, Martin et al., Immunospecific Targeting of Liposomes to Cells, Biochemistry, 1981, 20, p. 4229-4238, the disclosure of which is specifically incorporated herein by reference. Antimyosin has potential for NMR imaging of infarcted heart muscle. Moreover preparation of antifibrin has recently been reported; Hui et al., Monoclonal Antibodies to a Synthetic Fibrin-Like Peptide Bind to Human Fibrin but not Fibrinogen, Science, 1983, 222 p. 1129-1131. This antibody would be expected to concentrate at the sites of blood clots, where fibrin has been formed. Vesicle agent carriers attached to antifibrin could provide NMR contrast for imaging clots and thrombin in blood vessels. There are, however, other surface modifications which provide for cell recognition that are known to alter the biodistribution of the vesicles. For example, carbohydrate receptor analogues bound to the vesicle surface have been shown to target vesicles. (Mauk, et al., Targeting of Lipid Vesicles: Specificity of Carbohydrate Receptor Analogues for Leukocytes in Mice, Proc. Nat'l. Acad. Sci. USA 77, 4430-4434 (1980); Mauk, et al., Vesicle Targeting: Timed Release for Leukocytes in Mice by Subcutaneous Injection, Science 207, 309-311 (1980).) Such targeting by surface modifications are directly applicable for altering the biodistribution of paramagnetic ion.

20 Claims

1. A nuclear magnetic resonance imaging contrast agent for scanning tissue comprising vesicles with paramagnetic material encapsulated therein, said vesicles being formulated with an agent to promote vesicle stability for a sufficient time to allow biodistribution of said vesicles for scanning of said tissue and formulated to permit adequate water proton exchange across the vesicle bilayer to provide contrast for scanning said tissue.
2. A contrast agent according to claim 1 in which the vesicles are formulated with cholesterol.
3. A contrast agent according to claim 1 or 2 in which antibodies, carbohydrates or other cell recognition targeting agents are attached to the vesicles to provide specific targeting.
4. A contrast agent according to claim 3 in which the targeting agent is the antibody antimyosin.
5. A contrast agent according to claim 3 in which the targeting agent is the antibody antifibrin.
6. A contrast agent according to any one of the preceding claims in which the paramagnetic material is present in a concentration of at least approximately 50mM.
7. A contrasting agent according to any one of the preceding claims in which the paramagnetic material is present in a concentration of from about 50mM to approximately 1M.
8. A contrast agent according to any one of the preceding claims in which the paramagnetic material is a salt of a transition metal or the lanthanide or actinide series of the periodic table.
9. A contrast agent according to claim 8 in which paramagnetic material is a salt of a paramagnetic ion selected from manganese, copper, gadolinium, erbium, chromium, iron, cobalt and nickel.
10. A contrast agent according to claim 8 or 9 in which the paramagnetic material is a paramagnetic compound of a paramagnetic ion and a chelate.
11. A contrast agent according to any one of claims 1 to 7 in which the paramagnetic material is a paramagnetic compound including a stable free radical.
12. A contrast agent according to any one of the preceding claims in which the efficiency of the paramagnetic material is enhanced by the addition of a charged polymer thereto.
13. A contrast agent according to claim 12 in which the charged polymer is poly-L-lysine.

14. A contrast agent according to any one of claims 1 to 7 in which the paramagnetic material is a paramagnetic ion associated with a chelate.
15. A contrast agent according to claim 12 in which the paramagnetic material is Gd-DTPA.
16. A contrast agent according to any one of the preceding claims in which the scanning tissue is tumour tissue.
17. A contrast agent according to any one of the preceding claims where the paramagnetic material is associated with a chelating agent bound to the surface of the vesicles.

Revendications

1. Agent de contraste pour la formation d'images par résonance magnétique nucléaire pour balayer les tissus comprenant des vésicules dans lesquels est encapsulée une matière paramagnétique, lesdits vésicules étant formulées avec un agent pour promouvoir la stabilité des vésicules pendant une durée suffisante pour permettre une biodistribution desdites vésicules pour le balayage desdits tissus et formulées de manière à permettre un échange eau-protons adéquat à travers la bicouche de la vésicule pour fournir un contraste pour le balayage desdits tissus.
2. Agent de contraste selon la revendication 1 dans lequel les vésicules sont formulées avec du cholestérol.
3. Agent de contraste selon la revendication 1 ou 2 dans lequel des anticorps, des hydrates de carbone ou d'autres agents de ciblage pour reconnaissance des cellules sont attachés aux vésicules pour fournir un ciblage spécifique.
4. Agent de contraste selon la revendication 3 dans lequel l'agent de ciblage est l'anticorps antimyosine.
5. Agent de contraste selon la revendication 3 dans lequel l'agent de ciblage est l'anticorps antifibrine.
6. Agent de contraste selon l'une quelconque des revendications précédentes dans lequel la matière paramagnétique est présente en une concentration d'au moins environ 50 mM.
7. Agent de contraste selon l'une quelconque des revendications précédentes dans lequel la matière paramagnétique est présente en une concentration allant d'environ 50 mM à environ 1 M.
8. Agent de contraste selon l'une quelconque des revendications précédentes dans lequel la matière paramagnétique est un sel d'un métal de transition de la série des lanthanides ou des actinides de la classification périodique des éléments.
9. Agent de contraste selon la revendication 8 dans lequel la matière paramagnétique est un sel d'un ion paramagnétique choisi parmi le manganèse, le cuivre, le gadolinium, l'erbium, le chrome, le fer, le cobalt et le nickel.
10. Agent de contraste selon la revendication 8 ou 9 dans lequel la matière paramagnétique est un composé paramagnétique d'un ion paramagnétique et d'un chélate.
11. Agent de contraste selon l'une quelconque des revendications 1 à 7 dans lequel la matière paramagnétique est un composé paramagnétique incluant un radical libre stable.
12. Agent de contraste selon l'une quelconque des revendications précédentes dans lequel l'efficacité de la matière paramagnétique est accrue par l'addition d'un polymère chargé.
13. Agent de contraste selon la revendication 12 dans lequel le polymère chargé est la poly-L-lysine.
14. Agent de contraste selon l'une quelconque des revendications 1 à 7 dans lequel la matière paramagnétique

tique est un ion paramagnétique associé à un chélate.

15. Agent de contraste selon la revendication 12 dans lequel la matière paramagnétique est le Gd-DTPA.

5 16. Agent de contraste selon l'une quelconque des revendications précédentes dans lequel le tissu balayé est un tissu tumoral.

17. Agent de contraste selon l'une quelconque des revendications précédentes dans lequel la matière paramagnétique est associée à un agent de chélation lié à la surface des vésicules.

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Ansprüche

15 1. Kontrastmittel zur Bilderzeugung mittels magnetischer Kernresonanz zum Abtasten von Gewebe, welches Bläschen mit in ihnen eingekapseltem, paramagnetischem Material enthält, wobei diese Bläschen mit einem Mittel zur Förderung der Bläschenstabilität während einer für die Bioverteilung dieser Bläschen ausreichenden Zeit formuliert sind, damit das Gewebe abgetastet werden kann, und welches so formuliert ist, daß ein adäquater Wasserprotonenaustausch durch die Bläschendoppelschicht möglich ist, um den Kontrast für das Abtasten des Gewebes zu besorgen.

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2. Kontrastmittel nach Anspruch 1, wobei die Bläschen mit Cholesterin formuliert sind.

3. Kontrastmittel nach Anspruch 1 oder 2, wobei Antikörper, Kohlenhydrate oder andere auf die Zellerkennung gerichtete Mittel an den Bläschen angebracht sind, um ein spezifisches Aufspüren zu gestatten.

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4. Kontrastmittel nach Anspruch 3, wobei der Antikörper Antimyosin das Aufspürmittel darstellt.

5. Kontrastmittel nach Anspruch 3, wobei der Antikörper Antifibrin das Aufspürmittel darstellt.

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6. Kontrastmittel nach einem der vorhergehenden Ansprüche, wobei die Konzentration des paramagnetischen Materials mindestens annähernd 50 mM beträgt.

7. Kontrastmittel nach einem der vorhergehenden Ansprüche, wobei die Konzentration des paramagnetischen Materials etwa 50mM bis annähernd 1M beträgt.

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8. Kontrastmittel nach einem der vorhergehenden Ansprüche, wobei das paramagnetische Material ein Salz eines Übergangsmetall oder aus der Lanthanid- oder Actinidreihe des Periodensystems ist.

9. Kontrastmittel nach Anspruch 8, wobei das paramagnetische Material ein Salz eines paramagnetischen Ions ausgewählt aus Mangan, Kupfer, Gadolinium, Erbium, Chrom, Eisen, Kobalt und Nickel ist.

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10. Kontrastmittel nach Anspruch 8 oder 9, wobei das paramagnetische Material eine paramagnetische Verbindung aus einem paramagnetischen Ion und einem Chelat ist.

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11. Kontrastmittel nach einem der Ansprüche 1 bis 7, wobei das paramagnetische Material eine paramagnetische Verbindung ist, welche ein stabiles, freies Radikal enthält.

12. Kontrastmittel nach einem der vorhergehenden Ansprüche, wobei die Wirksamkeit des paramagnetischen Materials durch die Zugabe eines geladenen Kunststoffes verstärkt ist.

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13. Kontrastmittel nach Anspruch 12, wobei der geladene Kunststoff Poly-L-lysin ist.

14. Kontrastmittel nach einem der vorhergehenden Ansprüche 1 bis 7, wobei das paramagnetische Material ein mit einem Chelat assoziiertes paramagnetisches Ion ist.

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15. Kontrastmittel nach Anspruch 12, wobei das paramagnetische Material Gd-DTPA ist.

16. Kontrastmittel nach einem der vorhergehenden Ansprüche, wobei das abzutastende Gewebe ein

Tumorgewebe ist.

17. Kontrastmittel nach einem der vorhergehenden Ansprüche, wobei das paramagnetische Material mit einem chelatbildenden Mittel, welches an der Oberfläche der Bläschen gebunden ist, assoziiert ist.

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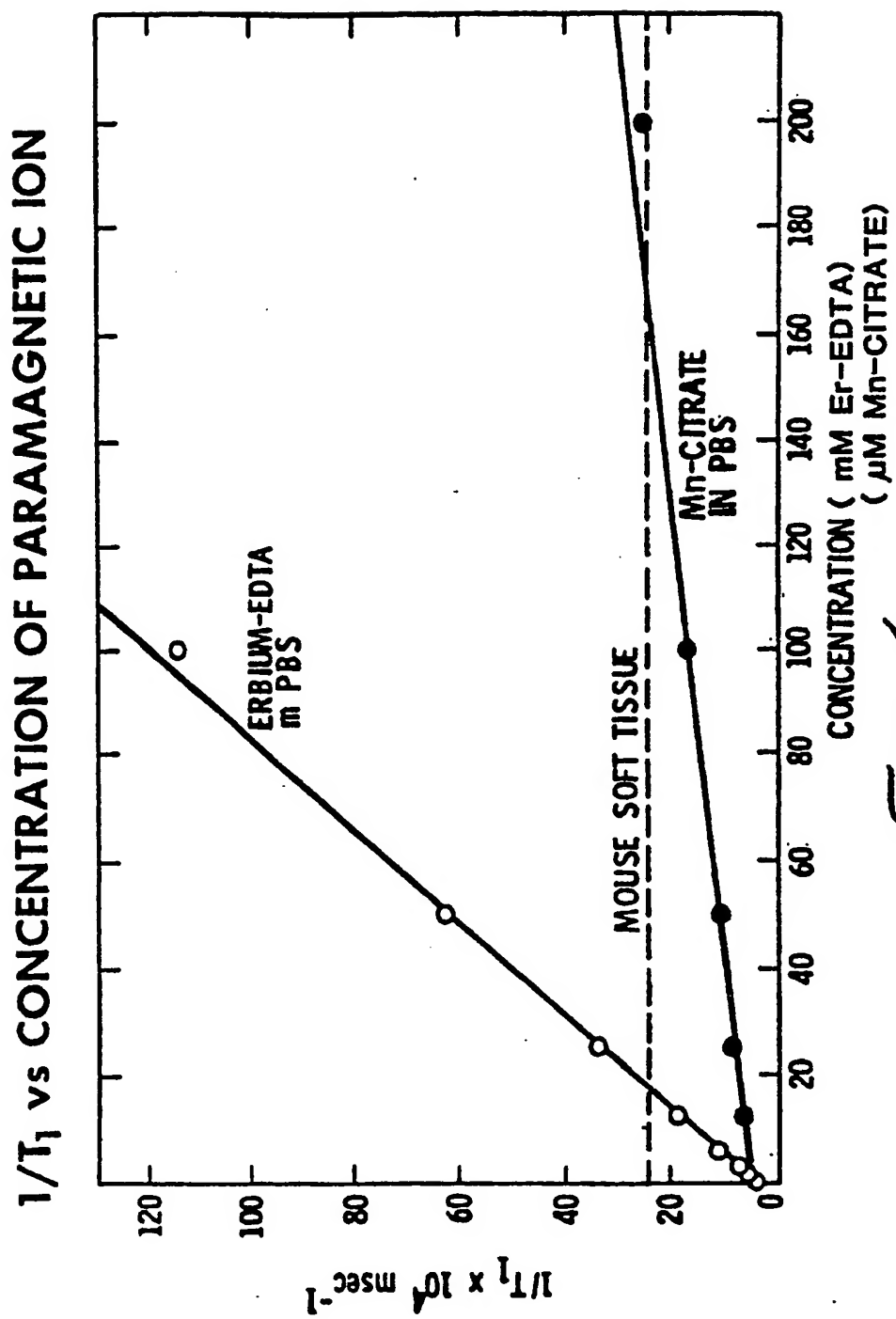


FIG. 1.

20 MHz RELAXATION RATES
38°C

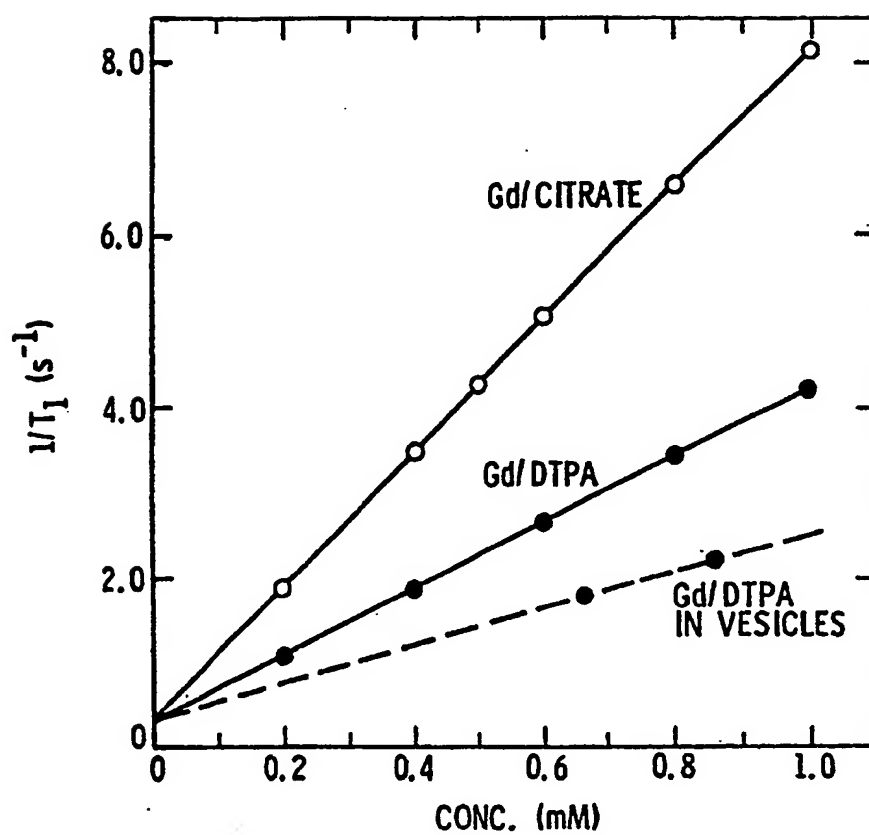


FIG. 2.

$1/T_1$ AND $1/T_2$ vs ENCAPSULATED [Gd/DPTA]

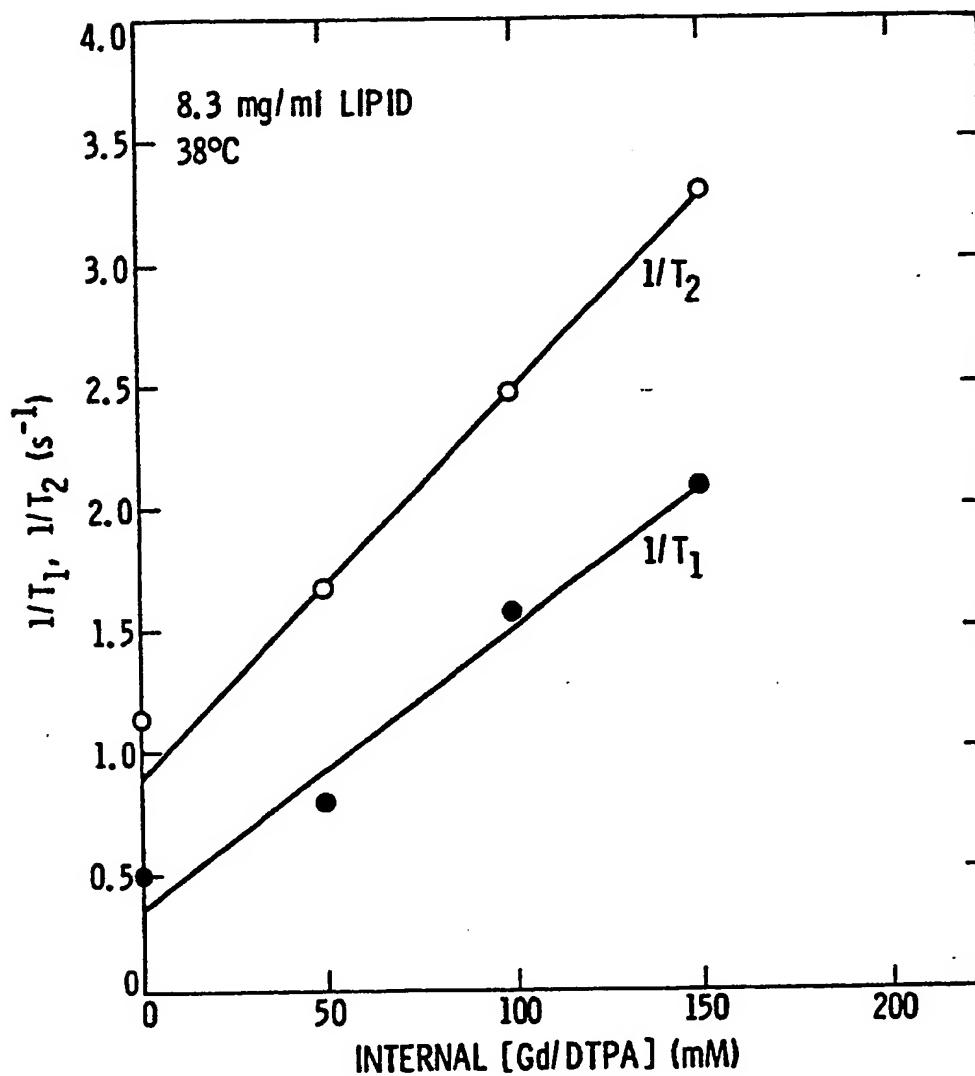


FIG. 3.

MOUSE ORGAN RELAXATION TIMES RARE EARTH/DTPA COMPLEXES

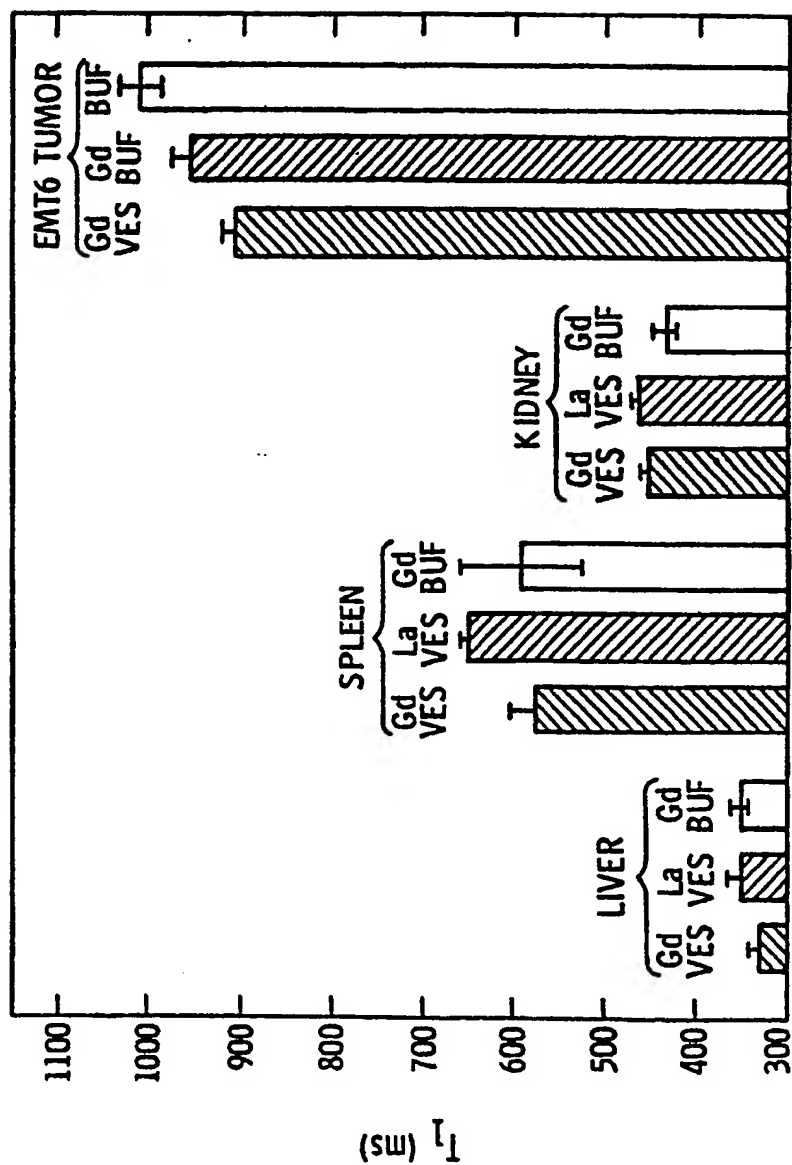


FIG. 4.

**NMR RELAXATION RATES FOR 2.0 mM
Gd/DTPA WITH ADDED POLY LYSINE; 38°C**

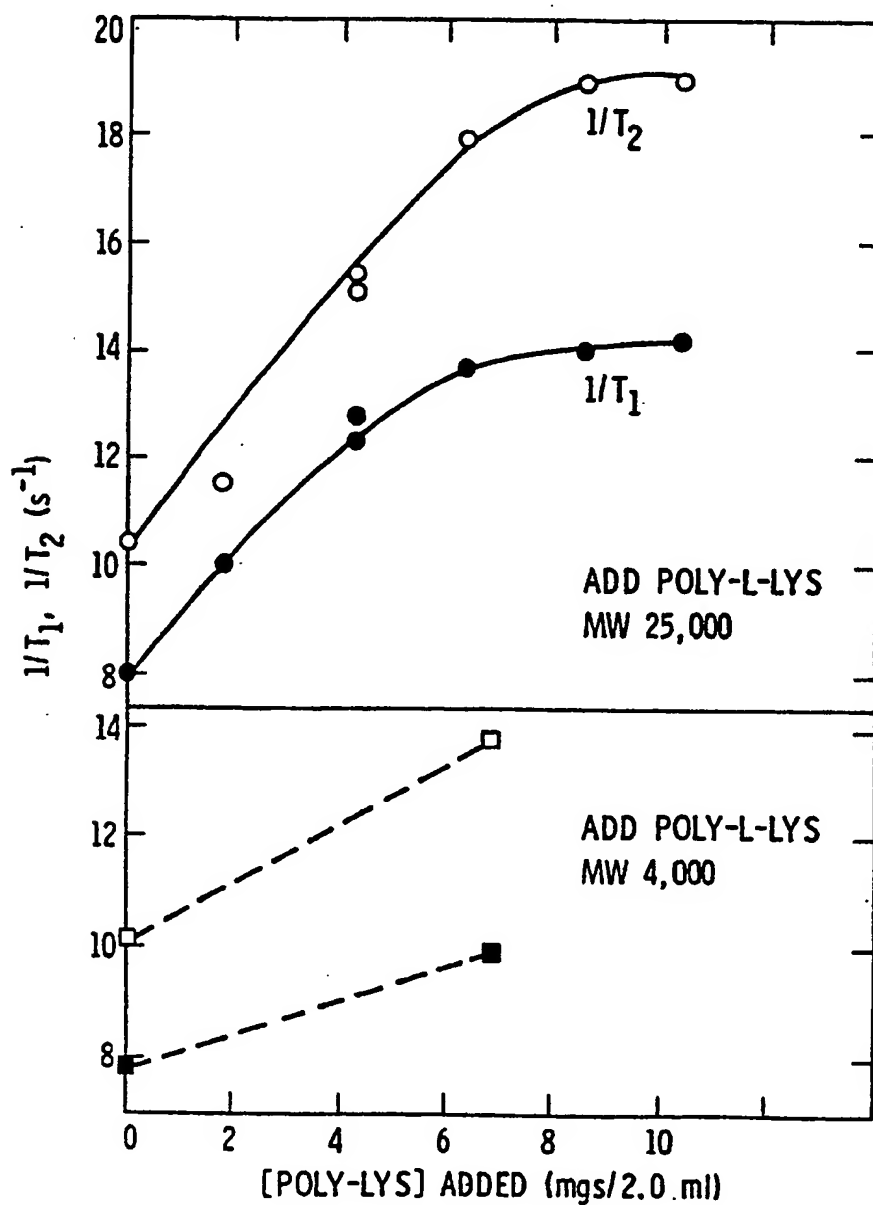


FIG. 5.

TIME COURSE OF RELAXATION EFFECT ON EMT6 TUMORS

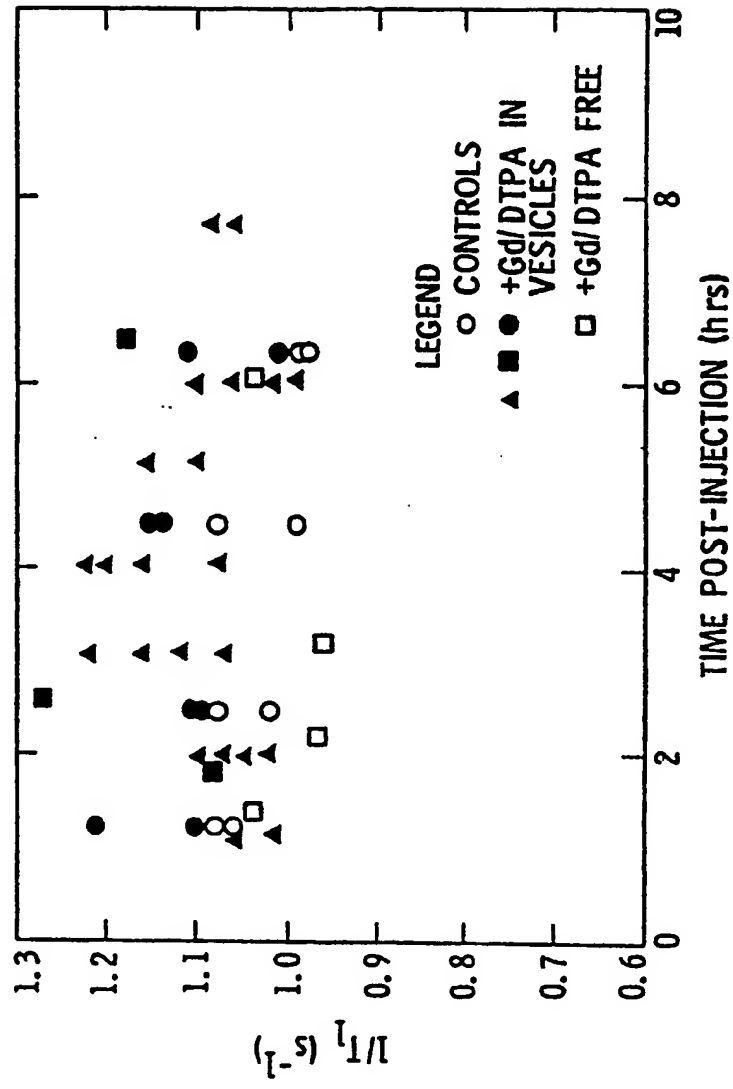


FIG. 6.